THE ANALYSIS OF A CASE OF CONTINUOUS VARIATION IN DROSOPHILA BY A STUDY OF ITS LINKAGE RELATIONS

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I. INTRODUCTION

HARDLY had the principles of Mendelism been worked out in one species of plant than apparent exceptions to these principles were discovered. Mendel's own case of the breeding true of species hybrids in *Hieracium* was the first of these, and since 1900 others have been reported.

Further analysis has shown that many of these early cases are readily interpreted on Mendelian principles, while for other exceptions, like that of *Hieracium,* for instance, the true explanation has been found without in any way coming into conflict with Mendelism.

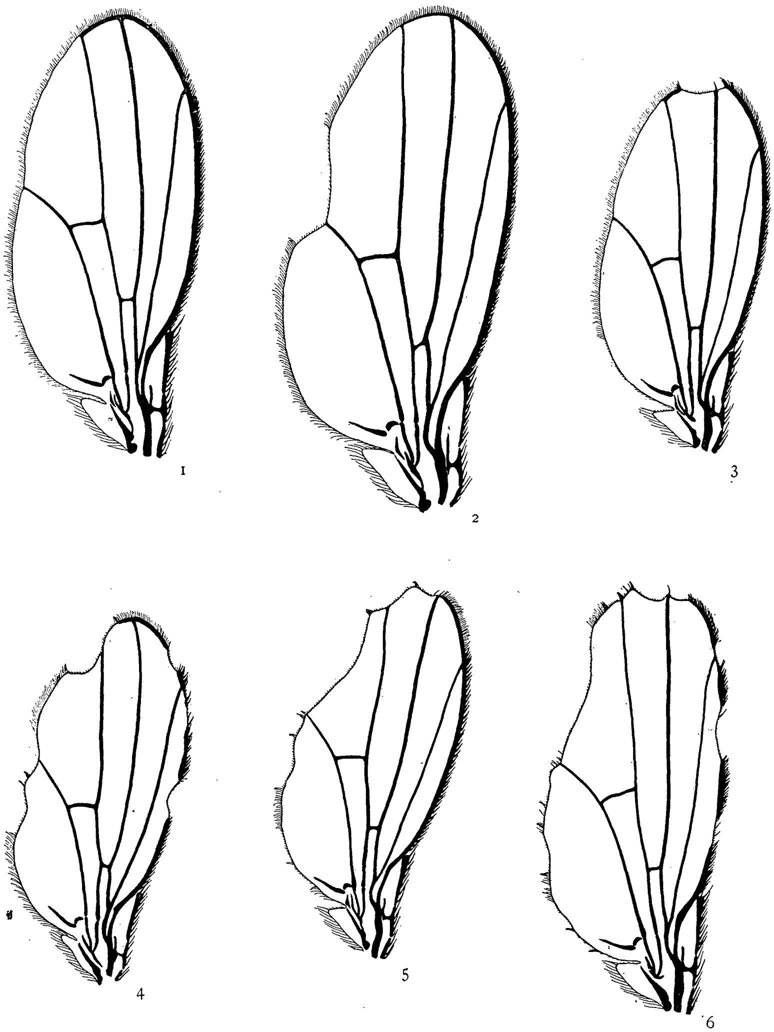
The masking of a Mendelian ratio may be effected in many ways, and some of the most important of the recent work in genetics has dealt with this problem. Among the conditions so far brought to light may be mentioned the following:

(*a*) *Multiple Factors.*—Recent papers by MacDowell (1914) and Shull (1914) have discussed at length the literature and history of this subject. In brief, the work that has been done shows that in both animals and plants the production of certain characters is brought about through the action of two or more independently Mendelizing pairs of genes that have similar effects on the developing organism. If the effect of these genes is cumulative, so that the character is more or less produced according to the number of dominant genes present, the type of inheritance known as blended inheritance is produced. If the effect is not cumulative, the recessive character does not appear with the frequency of 1:3, but with the frequency of 1:15, 1:63, etc., according to the number of pairs of genes concerned.

(*b*) *The Effect of the Environment.*—A typical case of this sort is reported by Baur (1912). In crossing a dark red to a red strain of *Antirrhinum,* a complete series between the red and the dark red appeared in the F_2 generation; the effect of light on the plants was such that plants that had developed in a bright light had a darker color than those that had developed in a less intense light. The analysis of the F_3 generation, however, proved conclusively that one fourth of the F_2 plants had been homozygous dark reds, one fourth had been homozygous red, and two fourths had been heterozygotes. Morgan (1912*a*) has described a case in *Drosophila* in which moisture conditions in the bottle in which the flies are developing determine to a certain extent whether or not certain

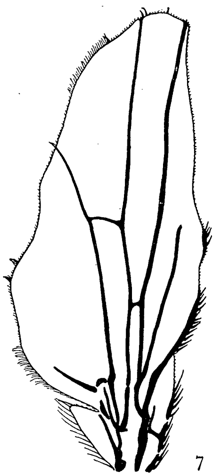
characters shall appear; and Hoge (1914) has shown that certain temperatures are necessary for the development of reduplicated legs in *Drosophila.* Other examples may be found in the literature of genetics.

(*c*) *Lethal Characters.*—There have been reported several instances in recent years of animals and plants which are unable to live if homozygous for certain genes. The



case of yellow mice, Baur's *Aurea*-strain of *Antirrhinum* (Baur, 1912) and the modified sex-ratios in *Drosophila* reported by Morgan (1912*d*) are examples of this phenomenon.

The object of the present paper is to describe a case of inheritance in *Drosophila* that for some years seemed to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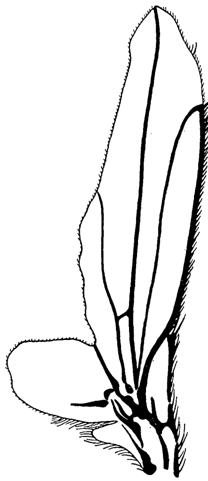
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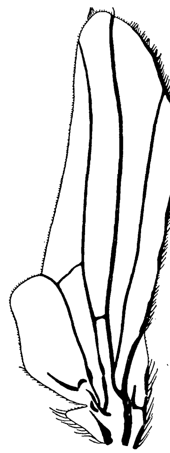
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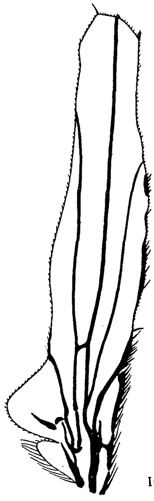
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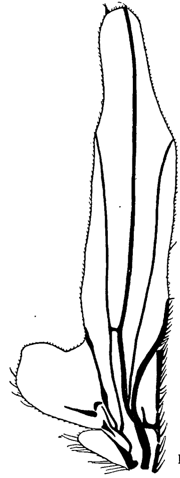
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defy Mendelian analysis. Though all the details of the case have not been worked out, enough has been done to show that it is brought about by factors which segregate in the ordinary Mendelian fashion, and that the difficulties which it still presents are not opposed to that hypothesis.

The case under consideration is that of Beaded wings, which, according to Morgan (1911*a*), first appeared in May, 1910, among flies that had been exposed during part of their early life to radium rays.

The appearance of these wings can best be understood from the figures (Figs. 1–12), which represent a few of the forms that may appear in a stock culture. All gradations may be found between wings perfectly normal and mere strips, such as shown by Figure 11.

In the early days of its history, according to Morgan, the Beaded-winged flies did not breed true, but for many generations produced many normal-winged offspring. At the time when I took up the experiment, however, the stock bred almost 100 per cent. pure; that is, almost every fly hatched had wings more or less Beaded. I have at present a strain which breeds true, throwing only Beaded-winged offspring, and most of the offspring have the Beading in an extreme form. Most of my work has been done with this stock.

II. THE GERMINAL CONSTITUTION OF BEADED FLIES

A. CROSSES BETWEEN BEADED AND WILD FLIES

1. *Behavior in First Generation*

When a Beaded fly is mated to a normal fly of a normal Wild stock, a considerable number of flies with Beaded wings usually appears in the first generation (F_1). The percentage is not constant, but varies between zero and about fifty per cent. (See Table I.) From Chart 1, it appears possible that the average percentage of Beaded-winged offspring per pair is near 10–15 per cent. or else near 30–35 per cent. of the total offspring. The exact

average is 25.5 per cent. That there is a bimodal curve produced may perhaps not be significant, as will appear

TABLE I

CROSSES OF BEADED TO WILD FLIES, SHOWING PERCENTAGES OF BEADED-WINGED OFFSPRING

	Per Cent. of Flies with Beaded Wings										
	0–4.9	5–9.9	10–14.9	15–19.9	20–24.9	25–29.9	30–34.9	35–39.9	40–44.9	45–49.9	50–100
Number of broods giving this percentage	1	5	10	6	7	7	14	3	2	2	2
Average size of brood	203	172	148	120	180	113	101	95	130	50	73

from the following facts, although later evidence will show that it very possibly is significant.

The per cent. of Beaded-winged offspring given by one pair (Beaded × Wild) may vary at different times and

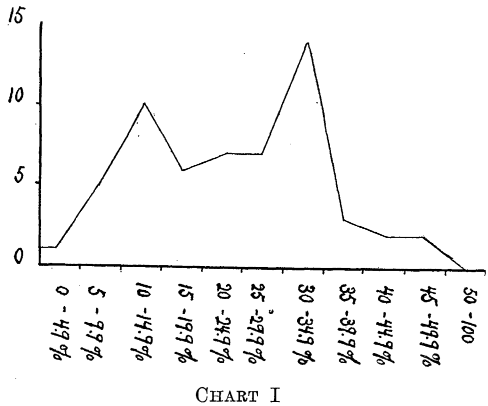


CHART I

Numbers of broods giving certain percentages of Beaded-winged offspring in F_1 generation of Beaded × Wild. (See Table I).

under different conditions. For instance, if a pair are put into a bottle with food and are left there for ten days, and are then put into another bottle with fresh food and left another ten days, the percentage of Beaded-winged offspring will be different in the two broods. Table II gives the records of such tests. The first two were made

with single pairs. In the third case, a Beaded male was given four virgin females, so that although all the offspring have the same father, they come from four mothers. Inspection of this table shows that it is quite impossible to assign the parents of any one brood to any definite class based on the percentage of Beaded-winged offspring that they give.

TABLE II

DIFFERENT PERCENTAGES OF BEADED-WINGED OFFSPRING BY THE SAME PARENTS DURING TWO SEPARATE TEN-DAY PERIODS IN DIFFERENT BOTTLES

	First Ten Days		Second Ten Days	
	No. of Offspring	Per Cent. Beaded	No. of Offspring	Per Cent. Beaded
First pair	126	21	179	41
Second pair	117	7	146	22
One father ×4 mothers	389	20	301	28

Table II shows also that the parents gave a larger percentage of Beaded-winged offspring during the second ten days than during the first period. That this is a coincidence appears from Table III. Here it is shown from the records of fifty broods chosen at random, that

TABLE III

PERCENTAGES OF BEADED-WINGED FLIES IN THE FIRST COUNT OF A BROOD COMPARED WITH THOSE OF THE LAST COUNT (INTERVAL OF FROM EIGHT TO TEN DAYS). BASED ON COUNTS FROM FIFTY BROODS, CHOSEN AT RANDOM

First Count	Last Count	First Count	Last Count	First Count	Last Count	First Count	Last Count	First Count	Last Count
36	7	10	0	10	33	10	13	3	7
4	0	42	24	1	0	25	24	51	30
10	0	71	25	8	1	24	0	23	11
20	0	0	1	19	7	11	3	15	12
1	0	5	5	3	0	32	0	43	8
48	20	64	15	36	10	40	0	42	0
45	4	37	3	25	3	46	9	32	6
37	40	17	6	18	0	60	0	50	40
33	15	10	0	47	10	16	0	29	4
21	0	10	0	28	20	52	0	56	29

Larger percentage of Beaded-winged offspring the first count, 44 broods.
Larger percentage of Beaded-winged offspring the last count, 5 broods.
Equal percentage of Beaded-winged offspring both counts, 1 brood.

the counts made in the first few days after the flies of any brood begin to hatch show almost invariably a very much larger percentage of Beaded-winged offspring than do the last counts made. This fact will be considered at some length in the section on environmental effects.

Enough has been said, at least, to show that, whether the results here described are genetic or environmental effects, the F_1 generation is remarkably inconstant with reference to the percentage of Beaded-winged offspring that appear. It is evident that this percentage can be readily altered by (1) changing the length of the period

CHART 2.

Bd ♂ × Wild ♀

daughters, 33% Bd — sons, 13% Bd

Bd ♂ × Wild ♀

daughters, 16% Bd — sons, 3% Bd

Bd ♂ × Wild ♀

daughters, 23% Bd — sons, 9% Bd

during which the brood is allowed to run; (2) by changing the parents from one bottle to another. Extensive studies of environmental effects have shown other ways in which the percentages can be altered, but of this we will treat later.

2. *Behavior in the Second Generation*

The question at once arises whether the Beaded and normal F_1 flies are alike genetically. To the solution of this problem two different breeding tests were applied: viz., matings of F_1 normal by normal, normal by Beaded, and Beaded by Beaded; and back crosses of both normal and Beaded to Wild stock. The results of these tests are given in Tables IV and V. These tables show that when

Beaded-winged flies of the F_1 generation are used as parents, more Beaded-winged young are produced than when normal-winged F_1 flies are used. This holds true for each

TABLE IV

MATINGS BETWEEN F_1 FLIES OF THE CROSS BEADED BY WILD, SHOWING PERCENTAGES OF BEADED OFFSPRING IN INDIVIDUAL BROODS

	Per Cent. of Flies Beaded													
	0–4.9	5–9.9	10–14.9	15–19.9	20–24.9	25–29.9	30–34.9	35–39.9	40–44.9	45–49.9	50–54.9	55–59.9	60–64.9	65–69.9
Normal ×Normal........	8	2	2	...	...	1	...	...	1	1	...	...	...	...
Normal ×Beaded........	1	...	...	...	...	1	...	3	1	...	2	...	...	...
Beaded ×Beaded........	...	...	...	...	...	...	...	...	...	1	2	1	1	1

TABLE V

BACK-CROSSES TO WILD OF F_1 FLIES OF THE CROSS BEADED × WILD, SHOWING PERCENTAGES OF BEADED OFFSPRING IN INDIVIDUAL BROODS

	Percentage of Offspring Beaded									
	0–4.9	5–9.9	10–14.9	15–19.9	20–24.9	25–29.9	30–34.9	35–39.9	40–44.9	45–50
Normal ×Wild..............	9	....	....	....	....	....	....	....	....	....
Beaded ×Wild..............	3	7	5	6	3	1	2	1	0	2

of the five crosses shown in the two tables. Normal-winged F_1 flies do, however, have some Beaded-winged offspring, both when mated among themselves, and also, though more rarely, when back crossed to Wild.

These F_2 and back-cross results give little satisfaction at first sight to the student of Mendelism. If we suppose that there is one gene on which the Beaded condition depends, and that it is partially dominant, then Beaded

TABLE VI

BEADED AND NORMAL OFFSPRING BY SEXES WHEN ONE PARENT IS BEADED AND THE OTHER WILD

	Beaded ♀♀	Normal ♀♀	Beaded ♂♂	Normal ♂♂	Per Cent. ♀♀ Bd.	Per Cent. ♂♂ Bd.
Father Beaded..	1,246	4,488	948	4,481	21.7	17.5
Mother Beaded .	894	2,959	1,139	2,684	23.2	29.8

and normal F_1 flies should give the same results when used as parents. Or if we were dealing here with a case like the "yellow mouse" case, in which homozygous yellows do not exist: that is, if homozygous "Beadeds" do not exist, then one quarter of the flies produced by two Beaded parents from the stock should be normal. But as was said before, the stock breeds true, every fly produced having Beaded wings.

It may be noted that a pair of F_1 normal flies usually produce less than 10 per cent. of Beaded offspring. If these normal flies carried a recessive gene for Beadedness, they should produce twenty-five per cent. Beaded offspring. The Beaded F_1 offspring, on the other hand, though they produced in all cases more than twenty-five per cent., did not produce 75 per cent. Beaded offspring, as they should have done if a single dominant gene for Beaded wings were heterozygous in them.

3. *Behavior in Third and Fourth Generations*

Beaded offspring, that appeared in the F_1 generation of the cross Beaded × Wild, were back crossed to Wild. The process was again repeated with the Beaded offspring that appeared, till four generations had been produced. The results of this test are given in Tables VII and VIII and in Chart 4.

A striking result is that an F_1 Beaded fly or even a fly of later generations heterozygous for Beaded wings some-

TABLE VII

Repeated Back-crosses of Beaded-winged Flies from the Cross Beaded by Wild to Wild Stock to Show Percentages of Beaded-winged Offspring. See Chart IV

	Family 1		Family 2		Family 3		Family 4		Family 5		Total	
	No. of Offspring	% Bd.	No. of Offspring	% Bd.	No. of Offspring	% Bd.	No. of Offspring	% Bd.	No. of Offspring	% Bd.	No. of Offspring	% Bd.
Generation 1	86	25.6	460	28.9	690	23.2	48	4.2	82	15.9	1,266	23.8
Generation 2	226	25.7	1,711	19.3	646	15.9	137	1.5	314	7.6	3,034	17.4
Generation 3	515	20.8	2,512	24.6	2,241	19.3	441	1.8	319	16.3	6,038	21.9
Generation 4	135	8.9	196	24.0	....	....	297	4.0	132	25.0	760	13.7

TABLE VIII

NORMAL FEMALES FROM FAMILY 2, GENERATION 2, BACK-CROSSED TO WILD MALES, SHOWING PERCENTAGES OF BEADED-WINGED OFFSPRING

	Beaded	Normal	% Bd.
Type X	2	1,040	.02
Type Y	50	342	15.3

times has as large a percentage of Beaded-winged offspring when mated to Wild, as does a fly direct from pure Beaded stock when mated to Wild, though a comparison of Tables I and V shows that this is not the usual occurrence. This suggests at once the action of a lethal gene (Morgan, 1912*b*). Morgan has shown that in a certain stock of *Drosophila* there are twice as many females as males in the offspring of one half the females. No matter to what male such a female be mated, her daughters are twice as numerous as her sons, and one half of her daughters also repeat this phenomenon, and one half of the daughters of these again. This fact finds its explanation in the assumption that there is in one of the sex-chromosomes of such females a gene which prevents the development of any male which gets it.

Now if such a gene had the power of expressing itself as a dominant in those flies that carried it in the heterozygous condition, if, for example, it caused the wings to be Beaded, it would be possible to select such flies at sight, and these flies could then be depended upon to repeat the phenomenon. (Morgan accomplishes the same end by mating such flies to mutants carrying a gene with which the lethal gene shows close linkage, such as that for white-eyes. He then finds that the red-eyed females carry the lethal gene, unless, as rarely happens, a "cross-over" has occurred.)

Such a sex-linked lethal gene producing a dominant wing character has actually been found to occur in the case of a mutant which arose in the Beaded stock, and which will be discussed later. For the present we must note that if the lethal gene were not associated with sex, its presence could be detected by the absence of certain

expected ratios, or classes, or in some other peculiarity of genetic behavior. In the case before us, we found that the F_1 generation consisted of at least two types; viz., Beaded and not-Beaded flies. These were shown to differ genetically. To obtain such a result must mean that at least one of the parents was heterozygous in at least one gene. This result is however a fairly constant one; and by virtue of the long-continued inbreeding of the Beaded stock this heterozygosity must surely have been weeded out before now if there were no serious hindrance to homozygosity. The classic example of this sort of effect is that of the yellow mice.

But the development of Beaded wings can not be brought about by the action of a *single* lethal gene, for if this were true it would be impossible to obtain a stock of Beaded flies that would breed true, and yet such a stock, as has already been said, is the one from which these very crosses derive their Beaded ancestors. There must therefore be at least one pair of allelomorphs of which one member is effective in producing Beaded wings, and can exist in the homozygous condition and possibly also another pair of allelomorphs of which one member is a recessive lethal gene. We can explain many of the facts so far obtained on the supposition, that there are these two independently Mendelizing pairs of allelomorphs concerned in the production of Beaded wings. The pair containing the lethal gene we will call L (normal) and l (lethal); and the other pair B′ (Beaded) and b′ (normal). The occurrence of the two genes B′ and l in one individual usually causes such an individual to have Beaded wings, though Beaded-winged flies also occur which do not carry the lethal gene, but are homozygous for B′.

It should be possible then to isolate a stock of Beaded-winged flies not carrying this lethal factor, l. Such flies should give a much smaller percentage of Beaded-winged offspring in the F_1 generation of a cross with Wild stock (or perhaps none at all, if B′ were recessive), than would those flies carrying lL. Such a stock has not yet been ob-

tained, but occasionally a strain of Beaded flies is met with that gives only low percentages of Beaded-winged offspring. See, for instance, Family 4, Table VII. Possibly such a stock would not be recognized at once, especially if it were so affected by environmental conditions that even flies homozygous for the factor B′B′ sometimes had normal wings. Normal-winged flies, as will be pointed out in a later section of this paper, do very frequently appear in Beaded stock, but these flies when mated to each other appear to throw as many Beaded-winged offspring as do the Beaded-winged flies of the stock, and often 100 per cent. of their offspring have Beaded wings.

In this connection it will be of interest to recall that Chart 1, and Table I gave results that might be interpreted as evidence of the bimodal curve that should be expected if the above hypothesis is correct.

Normal females from the second generation of Family 2 were also back-crossed to Wild males. The results are given in Table VIII. Most of these normal females gave very few or no Beaded offspring (Type X) while two of them gave a considerable number of Beaded offspring (Type Y). The explanation here is perhaps that the type Y females were genetically like most of the Beaded females of an F_1 generation (on our hypothesis, B′ L b′ l) while the females of Type X were genetically lacking in the factors that are usually present in Beaded F_1 flies (*i. e.*, they were B′ L b′ L). That such an occurrence is not infrequent in *Drosophila* is seen in Table IV in which three broods out of fifteen raised from normal F_1 flies gave 25 per cent. or more of Beaded offspring though the other twelve broods gave less than fifteen per cent., and eight broods less than five per cent. of Beaded offspring. It seems certain therefore that there are two types of normal-winged offspring in the F_1 generation of the cross, Beaded by Wild; one of these is genetically like the Beaded flies of the same generation and the other is genetically different from its Beaded brothers and sisters.

Types X and Y have been found to occur in all of the

tests made of F_1 flies whether of matings to Wild stock or of matings to other mutants such as Black, Pink, Arc, Ebony, etc. Table XXVI shows these two types as they appeared in back crosses to normal Pink males of normal and Beaded females of the cross Pink Beaded by Wild. Here it was found that more of the normal than of the Beaded F_1 flies were of Type X, and conversely that more of the Beaded than of the normals were of Type Y.

It has not been possible to distinguish with certainty between these two types even by their offspring because of the large amount of fluctuation that occurs in the percentages of Beaded offspring. For example it would be difficult to say whether a fly giving five per cent. of its offspring Beaded would belong to Type X or Type Y.

It would be expected that Type Y would be given by those flies that carried both factors for Beaded, and Type X by those that lack the lethal factor, and it will be seen later that on the whole the evidence supports this view.

B. Crosses between Beaded Flies and Other Mutants

1. *The F_1 Generation*

If we examine the F_1 generation when Beaded flies are crossed to other mutants, *i. e.*, to flies of a stock that is perfectly normal so far as Beadedness is concerned, but which is unlike the normal Wild flies in some other wing character, or in eye color or body color, etc., we find an even greater amount of variability in the percentage of Beaded-winged offspring than in the F_1 generation of Beaded by Wild. (See Tables I and IX; also Charts 1 and 3.)

The details may be gathered from Table IX, where it can be seen that there is a certain specificity in the percentage of Beaded offspring that appear in any specific mating.

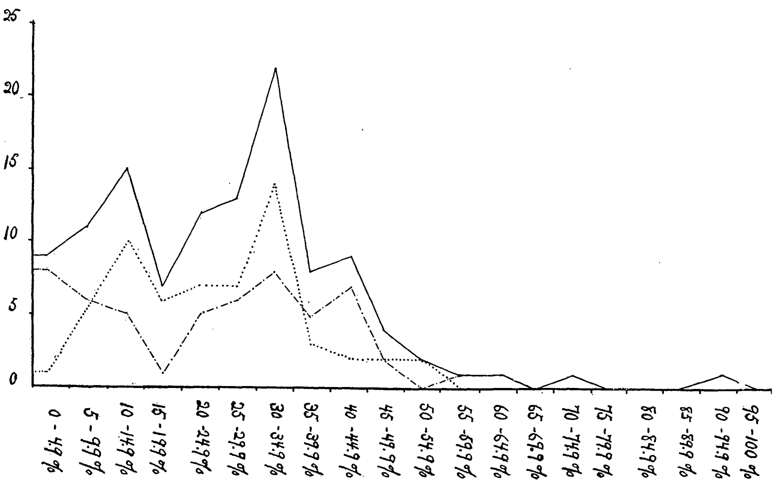
For instance, it appears that more of the offspring have Beaded wings if a cross is made with Vermilion-eyed flies than when Beadeds are mated to Pink-eyed

TABLE IX

THE PERCENTAGES OF BEADED-WINGED FLIES IN THE F_1 GENERATION OF CROSSES BETWEEN BEADED FLIES AND OTHER MUTANTS

Mutant Involved	Percentages																			
	0–4.9	5–9.9	10–14.9	15–19.9	20–24.9	25–29.9	30–34.9	35–39.9	40–44.9	45–49.9	50–54.9	55–59.9	60–64.9	65–69.9	70–74.9	75–79.9	80–84.9	85–89.9	90–94.9	95–100
Vermilion Yellow	..	1	..	..	..	1	1	..	..	1	..	..	..	..	..	..	..	..	..	..
Vermilion	..	..	..	..	1	..	..	1	4	..	..	..	..	..	..	..	..	..	..	..
Miniature Eosin	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..
White	..	..	..	..	..	1	4	3	1	1	..	..	..	..	..	..	..	..	..	..
Pink Beaded × White	2	..	1	..	..	2	..	..	1	..	..	..	..	..	..	..	..	..	..	..
Pink Beaded × Wild	4	4	4	1	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Pink	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Pink Ebony	..	..	..	..	1	1	..	..	1	..	..	..	1	..	..	..	..	..	..	..
Sepia	..	..	..	..	1	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..
Pink Black	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Black	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Purple Curved	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..
Truncate	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..
Vestigial	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..
Antlered	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..
Strap	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..
Total	8	6	5	1	5	6	8	5	7	2	..	1	1	..	1	..	..	..	1	..
Beaded × Wild (Table I)	1	5	10	6	7	7	14	3	2	2	2	..	..	..	..	..	..	..	..	..
Grand Total	9	11	15	7	12	13	22	8	9	4	2	1	1	..	1	..	..	..	1	..

CHART III



Distribution of broods giving certain percentages of Beaded-winged offspring in F_1 generation of Beaded × Normal (other Mutants or Wild). (See Table IX.)

flies, or more in the crosses with White-eyed flies than in those with Black body color. (In every case, where the contrary is not stated the flies are normal in other respects than the one named, *e. g.*, White-eyed flies in these crosses have Gray bodies and Long normal wings.)

No explanation of this specificity by the assumption of a segregation of factors in the germ cells appears to be available here, though such a possibility has not yet been ruled out, or can be ruled out till certain other phenomena are understood. The easiest way of "explaining" it is that the dominance of the genes for Beadedness varies in accordance with many other circumstances, among which are differences in the other genes present, such as those for Vermilion, White or Pink. Such an assumption as this, as will appear later, would seem to be fully in accord with the behavior of the genes for Beaded wings when in still different relationships.

It is assumed, then, for example, that the percentages of Beaded-winged flies in the F_1 generation of a cross between Beaded and White are higher than those in the F_1 generation of a cross between Beaded and Black, because the gene for Black is relatively to the gene for White eyes an inhibitor of Beadedness. It would appear as though a series might be made of the mutants of *Drosophila* beginning with those in which the genes for Beaded wings are most dominant and ending with those in which the Beaded genes are recessive. In order to construct such a series a large number of pairs would have to be made for each cross in order to determine the limits of variability of Beadedness for the cross concerned. The work would probably be greater than the value of the results obtained, and therefore the attempt has not been made to carry out this test. From what has been done incidentally in carrying out other experiments, it will be seen that in general the darker eye colors and body colors are associated with a low percentage of Beadedness in the F_1 generation, and the brighter colors with a higher percentage. This may, however, only be a coincidence.

2. *Linkage Relations*

(*a*) Sex Linkage

If in the crosses thus far described the sex of parents and offspring that show Beaded wings be considered, it may appear at first as though we may be dealing with a partially sex-linked gene. For it very frequently happens that when the mother is Beaded, and the father is normal (either of Wild stock or of some mutant stock not carrying Beadedness), more of the sons than of the daughters are Beaded. For example, in one such brood, there were 17 Beaded to 128 normal females, and 5 Beaded to 130 normal males, or 12 per cent. of the females and 3.5 per cent. of the males. Both of these examples were deliberately chosen because they were good examples of the phenomenon described. It would be possible to select from my records several examples of the reverse phenomenon, where Beaded females had more Beaded daughters than Beaded sons, and where Beaded males had more Beaded sons than Beaded daughters. Nevertheless, the records of all broods available have given the numbers shown in Table VI, where it appears that more sons are Beaded when only the mother is Beaded and more daughters when only the father is Beaded.

It may perhaps be significant, on the other hand, that when the mother is Beaded a *slightly larger percentage* of her daughters is Beaded than of the daughters of a Beaded male, while a *very much larger percentage* of her sons is Beaded than the sons of a Beaded male. In other words, it seems that the daughters are affected to approximately the same extent, whether they get their Beadedness from father or mother, while the sons are affected also by the mother, whether or not she carries Beadedness. This might mean that there is some gene in the sex chromosome that does not show except when other Beaded factors are present. That this is not the case will appear from Chart 2, which records three generations of flies in each of which the mother was normal (Wild)

and the father Beaded. This shows that although the father transmitted his Beadedness more to his daughters than to his sons, yet his Beaded sons also had the capacity to affect their daughters more than their sons, and these sons again repeated the phenomenon. Yet these males could not have received their X-chromosome from their father, unless non-disjunction (see Bridges, '13) had occurred. In fact, to produce the results here given non-disjunction must occur in one half the females of the Wild stock. Frequent tests with the Wild stock by practically all of the students in the laboratory make it certain that this is not the case. I also tested a considerable number of the females by mating them to sex-linked mutants and found no non-disjunction.

This apparent sex-linkage that does not follow the "ordinary rules" of sex-linkage must be left for the time being as one of the still unsolved problems. The only possibility of explanation that occurs to me is that the above-described effect would be produced if in the cytoplasm of the egg of the Beaded female something were present which is absent in the egg of the normal female, and to which the males are more responsive in their development than are the females. This suggestion has not a particle of cytological evidence to support it. Morgan (1912*d*) has suggested that the influence of cytoplasm may cause certain peculiar results obtained in crosses between Miniature-winged and Rudimentary-winged flies.

(*b*) Linkage to Sex-linked Genes

Matings of Beaded flies to flies with sex-linked characters, including Vermilion and Vermilion-yellow, have been made and the F_2 generation raised. No sign of linkage was observed. The F_2 figures are given in Tables X and XI. These cases definitely establish that there is no gene for Beaded wings in the X-chromosome.

Although no sex-linked gene for Beaded wings are known, there has arisen in the Beaded stock by mutation a fly with notched wings (Fig. 13) that proved to be

TABLE X

F_2 COUNTS FROM THE CROSS VERMILION ♀ × BEADED ♂

V.Bd. ♀	V.Bd. ♂	V.N. ♀	V.N. ♂	Red Bd. ♀	R.Bd. ♂	R.N. ♀	R.N. ♂
39	26	93	77	33	22	81	60

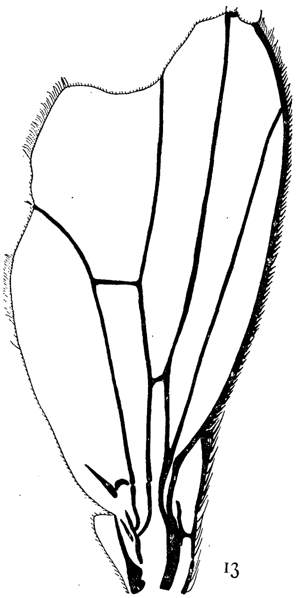
	% Bd.	Expected No. of Bd. if no coupling exists
Bd.V : N.V. = 65 : 170	27.6	65.4
Bd.Red : N.Red = 55 : 141	28.0	54.6
Bd.Total : N.Total = 120 : 311	27.8	(120)

TABLE XI

F_2 COUNTS FROM THE CROSS VERMILLION YELLOW × BEADED

Beaded Gray Red	Normal Gray Red	Beaded Gray Vermil.	Normal Gray Vermil.	Beaded Yellow Red	Normal Yellow Red	Beaded Yellow Vermil.	Normal Yellow Vermil.
165	227	35	34	41	34	50	88

	% Beaded	Expected No. of Bd. if no coupling exists
Bd.R. : N.R. = 206 : 261	44.1	201.7
Bd.V. : N.V. = 85 : 122	41.0	89.4
Bd.G. : N.G. = 200 : 261	43.4	199.1
Bd.Y. : N.Y. = 91 : 122	42.7	92.0
Bd.Total : N.Total = 291 : 383	43.2	(291.0)



13

caused by a dominant sex-linked factor lethal when homozygous. (See page 754.) It will be discussed under the name "Perfect Notched" and its peculiarities described in the section on "Mutation in Beaded Stock."

(c) Linkage to Second Chromosome Genes

For the reasons given, it seems certain that there is in the group of sex-linked genes no gene concerned in the production of Beaded wings. We shall later bring forward evidence to show that there is such a gene in the third chromosome group. (Sturtevant, 1913.) The crosses made with flies showing characters whose

genes are in the second chromosome are still perplexing; for while the second chromosome exerts an influence on the statistical results, as will be evident from the figures to be presented, the nature of this influence is not fully determined.

The second chromosome characters with which tests have been made are the wing characters, Arc, Curved, Vestigial, Antlered, and Strap,[1] the body color, Black; and the eye color, Purple.

Vestigial, Antlered, and Strap stand for wing characters of such a nature that it is not possible to distinguish Beaded-winged individuals if any of these other characters are also present. They are therefore of no use for determining whether or not there is a second chromosome gene for Beaded wings. The crosses between Beaded flies and flies with these characters do not especially interest us here. It may be said in passing, however, that in every case in the F_1 generation between these flies and Beaded flies, from 60 to 90 per cent. of the offspring had non-normal wings, and the author was put to serious straits to classify the new wing types that appeared. These were similar in all the crosses, however, and on the whole resembled Beaded wings.

In the F_2 generation, and in back crosses to Beaded Stock and to Vestigial Stock further complications arose with more new types of wings, including a new "mutation" which bred true from the start, and which will be discussed briefly and described under the name "Spread" in the section that concerns mutation.

We may now return to the crosses between Beaded flies and Black, or Purple, or Arc, or Curved. These crosses give results that can be used for the study of linkage, and they present in common a number of distinguishing

[1] Strap Wings is a mutant much resembling Extreme Beaded in appearance but its mode of inheritance has not yet been worked out. It may be that it actually is Beaded plus some at present unknown gene. Beadedness is suspected to occur also in Vestigial and Antlered stock. This may very likely be true since Strap and Antlered arose in Vestigial, and Vestigial in Beaded.

characteristics. Tables XII to XX give the results in systematic form.

TABLE XII

F_2 COUNTS OF THE CROSS BEADED ♀ × CURVED ♂

	Beaded Curved	Normal Curved	Beaded Straight	Normal Straight	Exp. Bd. Curved	Exp. Bd. Straight
Type 1.........	4	61	16	246	4	16
Type 2.........	2	25	18	105	3.6	16.4

One of the most striking characteristics of these second chromosome crosses is that the F_1 flies fall into two classes or possibly into three classes with reference to the offspring that they produce. These classes I have called Type 1, Type 2, and Type 3.

In Type 1 there is no linkage between Beaded wings and the second chromosome character, but Beaded-winged flies occur with equal frequency in all classes of

TABLE XIII

F_2 COUNTS OF THE CROSS BEADED ♀ × ARC ♂

	Beaded Arc	Normal Arc	Beaded Straight	Normal Straight	Exp. Bd. Arc	Exp. Bd. Straight
Type 1.........	39	200	184	970	38.+	185
Type 2.........	41	152	330	452	73	298

offspring. In Type 2 there is linkage of Beaded wings with the second chromosome characters, so that the Beaded wings appear more frequently in flies showing the characters of the Beaded parent. In Type 3, which occurs only a very few times and is not very marked except in Table XX, Beaded wings appear to a greater percentage in the offspring whose other characters are not those of the Beaded parent. (*I. e.*, "repulsion" occurs between the factor for Beadedness and that for the second chromosome character with which it entered the cross.) I do not wish to emphasize Type 3, but concerning the other two it is important to note that about one half of the F_1 flies seem to be of Type 1 and one half of Type 2.

In Type 2 in the cases here adduced the linkage is

strongest with the character Arc and weakest with Black. If there is a gene in the second chromosome which aids in producing Beaded wings, it seems probable that it is located nearer to Arc than to Black and on the side of Arc away from Black.

TABLE XIV

BACK-CROSSES OF F_1 BEADED-WINGED MALES OF THE CROSS BEADED ♂ × PURPLE CURVED ♀ TO PURPLE CURVED FEMALES OF NORMAL STOCK

	Beaded Pr. Curved	Normal Pr. Curved	Beaded Red Straight	Normal Red Straight	Exp. Nor. Bd. Pr. Cv.	Exp. Nor. Bd. R. Strt.
Type 1.........	15	125	15	154	13.6	16.4
Type 2.........	2	29	28	27	10.8	19.2

It remains to consider Type 1, and to find the reason for the existence in the F_1 generation of flies whose offspring show no linkage between Beadedness and second chromosome characters, and in the same brood, flies whose offspring do show such linkage. The most obvious "explanation" would be, of course, that the factor in the

TABLE XV

BACK-CROSSES OF F_1 BEADED-WINGED FEMALES OF THE CROSS BEADED ♂ × PURPLE CURVED ♀ TO PURPLE CURVED MALES OF NORMAL STOCK

Brood 2 is of Type 3 for Purple and of Type 1 for Curved.

	Bd. Pr. Cv.	N. Pr. Cv.	B. Pr. St.	N. Pr. St.	Bd. Red Cv.	N. R. Cv.	Bd. R. St.	N. R. St.
Brood 1.............	7	49	1	22	1	17	16	61
Brood 2.............	10	47	4	6	0	12	14	71

second chromosome was a "lethal" such as the factor l, described in an earlier section of this paper. If this were the case, there should be some flies in the Beaded stock homozygous for L, the normal allelomorph of this gene, and also for B′, *i. e.*, B′LB′L. These flies should have fewer Beaded offspring than those heterozygous for l, and none of these F_1 offspring should give linkage with second chromosome characters. As a matter of fact, in the F_2 results given in Table XVI for Beaded by Black, no linkage was observed; but this case is not good evidence, for it was made in the first attempts to solve the problem of Beaded wings, and I had not yet learned the

value of F_1 counts, matings in pairs, and back-crosses to normal. It stands however as the only evidence of its sort that I can give at present.

TABLE XVI

F_2 COUNTS OF THE CROSS BEADED ♀ × BLACK ♂

	Beaded Black	Normal Black	Beaded Gray	Normal Gray	Expected No. Bd. Bl.	Expected No. Bd. Gray
Type 1.........	19	375	82	1602	19	82

Type 3 is not easy to explain. There are no known cases of this sort elsewhere in *Drosophila* and I prefer not to attempt to answer this question at present.

TABLE XVII

BACK-CROSSES OF F_1 MALES OF THE CROSS BEADED ♂ × BLACK ♀ TO BLACK FEMALES OF NORMAL STOCK

	Beaded Black	Normal Black	Beaded Gray	Normal Gray	Expected No. Bd. Bl.	Expected No. Bd. Gray
Type 1.........	7	162	9	187	7.3	8.6
Type 2.........	5	110	40	97	20.5	24.5

In general, it may be noted that technical difficulties have disturbed the crosses with second chromosome characters. The wing character Arc is not always easy to recognize, as it is very often nearly normal in appearance. On the other hand, the flies with Curved wings, though always distinct, occasionally get "stuck up" with the food and in their bedraggled condition it can not always be determined whether or not the wings are Beaded as well as Curved. I was at first inclined to attribute the apparent coupling (which was discovered for Arc and for Curved before it was discovered for Black) to errors made in the counts.

As for Black, the F_1 and later generations give a much lower percentage of Beaded offspring than do most other crosses, and this necessitates raising large numbers of offspring. The results are, however, trustworthy when obtained.

The crosses with Purple-eyed flies presented no difficulties but ran smoothly aside from the fact that the purple-eyed flies had Curved wings, and as remarked above, Curved wings sometimes get bedraggled.

TABLE XVIII

BACK-CROSSES OF F_1 FEMALES OF THE CROSS BEADED ♂ × BLACK ♀ TO BLACK MALES OF NORMAL STOCK

	Beaded Black	Normal Black	Beaded Gray	Normal Gray	Expected No. Bd. Bl.	Expected No. Bd. Gray
Type 1.........	5	78	2	91	3.3	3.7
Type 2.........	5	457	28	493	15.5	17.5

(*d*) Linkage to Third Chromosome Genes

We have said tentatively that there was perhaps a non-sex-linked lethal gene for Beaded wings in the second chromosome, and that possibly the cytoplasm carried by the egg disposes males toward or away from Beadedness according to whether the fly that bore the egg was or was not Beaded. These relations are not securely determined, and the data are still incomplete. The relation of Beaded wings to characters whose genes are in the third chromosome is much clearer. All crosses that bear on this problem point to one fact, namely, that there is a gene for the production of Beaded wings in the third chromosome, and that this gene is very closely linked to Ebony, and very loosely linked to Pink. Tests have been made between Beaded and the third chromosome characters, Maroon, Sepia, and Pink eyes and Ebony body color.

TABLE XIX

F_2 COUNTS OF THE CROSS PINK BEADED ♂ × BLACK ♀

	Pink Bd. Bl.	Pink N. Bl.	Pink Bd. Gray	Pink N. Gray	Red Bd. Black	Red N. Black	Red Bd. Gray	Red N. Gray
Type 1 (?)	2	25	20	57	0	45	3	278

	Expected No. Bd. if no coupling occurs
Bd.Pink : N.Pink = 22 : 82....................	6
Bd.Red : N.Red = 3 : 323	19
Bd.Black : N.Black = 2 : 70....................	4
Bd.Gray : N.Gray = 23 : 335....................	21
Bd.Total : N.Total = 25 : 405	(25)

In the cross of Beaded by Maroon-eyed flies, 1,369 flies were raised in the F_2 generation. Fifty-seven of these flies had Beaded wings; only one of the Beaded-winged flies had Maroon eyes, while fifty-six were red-eyed. (See Table XXI.)

TABLE XX.

BACK-CROSSES OF F_1 FEMALES OF THE CROSS PINK BEADED ♂ × BLACK ♀ TO PINK BLACK MALES FROM NORMAL STOCK

	Pink Bd. Black	Pink N. Black	Pink Bd. Gray	Pink N. Gray	Red Bd. Black	Red N. Black	Red Bd. Gray	Red N. Gray
Type 1.....	5	98	8	113	5	92	1	122
Type 3.....	12	68	6	58	7	78	3	76
Totals ...	17	166	14	171	12	170	4	198

	Expected No. Beaded if no coupling occurs
Bd.Pink : N.Pink = 31 : 337..................	23
Bd.Red : N.Red = 16 : 368.....................	24
Bd.Black : N.Black = 29 : 336................	23
Bd.Gray : N.Gray = 18 : 369..................	24
Bd.Total : N.Total = 47 : 705................	(47)

F_1 males of the cross Sepia by Beaded were back-crossed to normal Sepia females. Inasmuch as cross-overs probably do not occur in the male (Morgan, 1912*c*), no Beaded Sepia flies should occur in the offspring of this cross. Table XXII shows that none occurred. The numbers are not large, but since they are entirely in accord with the other third chromosome results, it was not thought worth while to increase them. That apparent cross-overs may very rarely occur will appear possible when we consider the results of crossing Beaded by Pink, and the probable significance of the phenomenon will be considered.

TABLE XXI

F_2 RESULTS OF THE CROSS BEADED ♀ × MAROON ♂

Bd. Maroon	N. Maroon	Bd. Red	N. Red	Exp. Bd. N.	Exp. Bd. R.
1	318	56	994	13	44

In F_2 counts of the crosses involving Beaded and the body color Ebony, totaling 4,417, in which 1,205 Beaded-winged offspring occurred, not one had the body color Ebony, and only eleven had Pink eyes. Repeated attempts

to obtain Ebony flies with Beaded wings have failed. The possibility that for some "inherent peculiarity" an Ebony fly can not have Beaded wings has suggested itself,

TABLE XXII

BACK-CROSSES OF F_1 BEADED MALES OF THE CROSS BEADED ♂ × SEPIA ♀ TO SEPIA FEMALES OF NORMAL STOCK

Bd. Sepia	N. Sepia	Bd. Red	N. Red
0	134	9	132

and although this would seem very improbable, it may nevertheless be the fact. At any rate, it appears that Beadedness either depends on genes which in the presence of the Ebony body color are completely recessive, or that the third chromosome gene for Beadedness, B′, lies so close to that for Ebony that cross-overs are extremely rare even in the female.

TABLE XXIII

F_2 RESULTS OF THE CROSS BEADED ♀ × EBONY ♂

Beaded Ebony	Normal Ebony	Beaded Gray	Normal Gray
0	151	17	525

Very extensive experiments were carried out with Pink-eyed flies. The important facts brought out are presented in condensed form in Tables XIX, XX, and XXIV–XXIX.

In Table XXIV are shown the F_2 counts for Beaded by Pink Ebony. From the eleven Pink Beaded flies obtained a new stock was derived, which was "purified" by a few

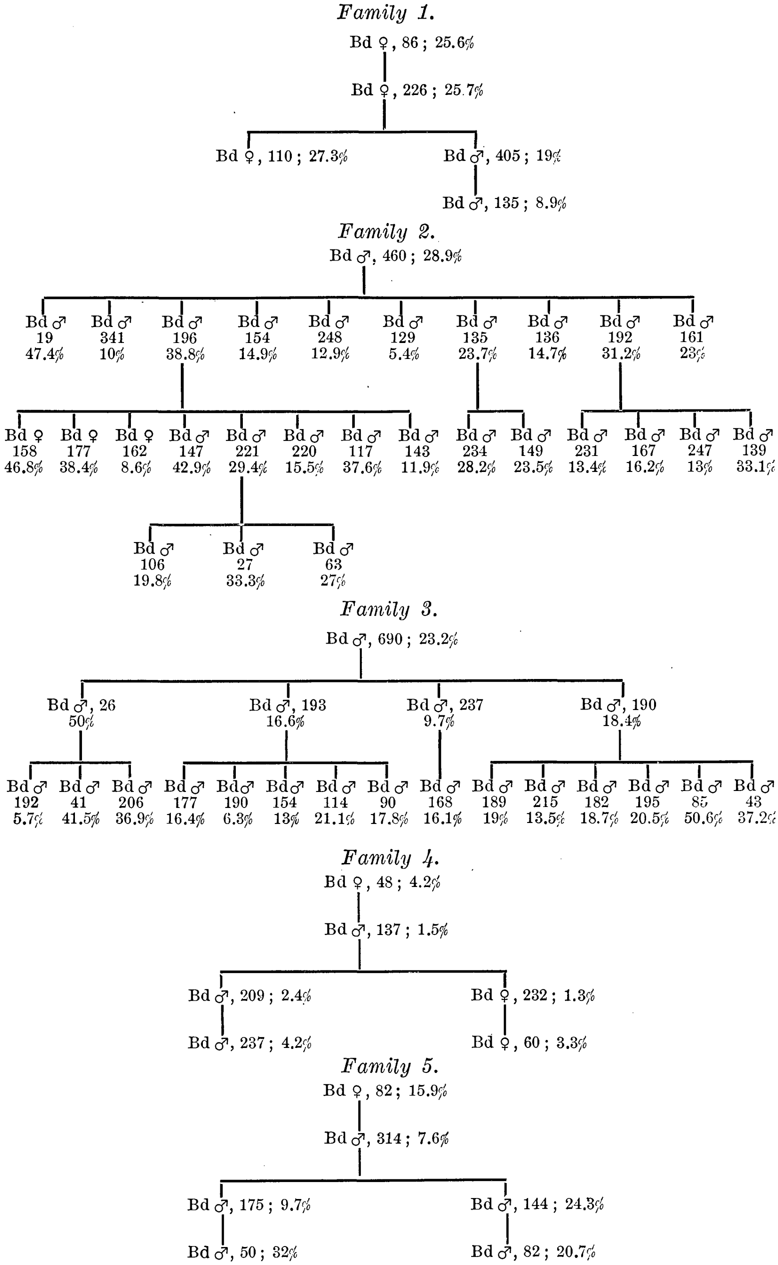
TABLE XXIV

F_2 RESULTS OF THE CROSS BEADED ♀ × PINK EBONY ♂

Bd. P. Eb.	N. P. Eb.	Bd. R. Eb.	N. R. Eb.	Bd. P. Gray	N. P. Gray	Bd. R. Gray	N. R. Gray
0	847	0	182	11	157	1,177	1,350

	Expected No. Bd. if no coupling occurs
Bd.Ebony : N.Ebony = 0 : 1029	328
Bd.Gray : N.Gray = 1188 : 1507	860
Bd.Pink : N.Pink = 11 : 1004	324
Bd.Red : N.Red = 1177 : 1532	864

CHART IV



Repeated back-crosses of Beaded × Wild in successive generations, showing sex of Beaded parent, number of offspring, and percentage of offspring with Beaded wings.

generations of selection, and now gives approximately 100 per cent. Beaded offspring, though no selection has been practised for nearly a year. This stock has been used in one series of crosses to supplement another series in which Pink and Beaded enter the cross from opposite parents. The results in each case are essentially similar, and show that when Beadedness enters with Red it comes

TABLE XXV

F_2 RESULTS OF THE CROSS PINK BEADED × WILD

Bd. Pink	Normal Pink	Beaded Red	Normal Red	Exp. No. Bd. P.	Exp. No. Bd. R.
171	213	366	964	120	417

out more with Red than with Pink. They show that in the F_1 female crossing over occurs almost independently of Pink, so that almost the same percentage of Beaded-winged individuals appears in each class, though usually the class that is similar to the Beaded parent is considerably the largest. In Table XXVII, however, a record is given in which a very considerable "repulsion" occurred, and the high Beaded class is not Pink Beaded, as is there expected, but Red Beaded. The results from back-crosses of the brothers of these females to Pink normal stock show that no mistake was made in recording the cross, which therefore, though somewhat surprising, must stand.

TABLE XXVI

BACK-CROSSES OF F_1 FEMALES OF THE CROSS PINK BEADED × WILD TO PINK MALES OF NORMAL STOCK

	Bd. Pink	N. Pink	Bd. Red	N. Red	Exp. No. Bd. P.	Exp. No. Bd. R.
Type X........	3	333	2	337	2.5	2.5
Type Y........	71	332	58	369	62.6	66.4
Total........	74	665	60	706	65	69

The tables show also that in the males, crossing over is of very rare occurrence, if, indeed, it occurs at all. The records show that out of 566 Beaded flies (Tables XXVII and XXIX) which occurred as the offspring of an F_1 male

back-crossed to Pink normal stock, six flies of the cross-over class appear. For reasons to be mentioned, it is improbable that these represent cross-overs, however, but rather they may be due perhaps either to the presence of the second chromosome gene, l, which usually does not manifest itself in the absence of the third chromosome gene, or to mutation, or to some unknown abnormality. Through carelessness only one of these males was tested

TABLE XXVII

BACK-CROSSES OF F_1 MALES OF THE CROSS PINK BEADED × WILD TO PINK FEMALES OF NORMAL STOCK

Bd. Pink	N. Pink	Bd. Red	N. Red	Exp. No. Bd. P.	Exp. No. Bd. R.
56	710	5	805	29	32

or used further in breeding. They were very slightly Beaded, and had only a very slight "nick" at the tip of the wing, even smaller than that shown in Fig. 3. The single Pink Beaded male mentioned in Table XXIX was mated to several females but was sterile. Another test is also possible, and was made as follows. Pink normal males and females from Table XXIX, which of course should not carry the third chromosome gene for Beaded

TABLE XXVIII

REPEATED BACK-CROSSES OF F_1 FLIES OF THE CROSS PINK BEADED × WILD TO PINK FLIES OF NORMAL STOCK

	Bd. Pink	N. Pink	Bd. Red	N. Red	Exp. No. Bd. P.	Exp. No. Bd. R.
F_1 ♀ ×Pink ♂. Type X..........	1	228	1	272	1	1
F_1 ♀ ×Pink ♂. Type Y...........	24	379	79	383	48	55
Total..........................	25	607	80	655	49	56
F_1 ♂ ×P ♀.......................	7	133	0	135	3.5	3.5

wings, were then mated together, and among their 374 offspring three males with slight "nicks" at the tip of their wings, exactly like those of the Pink Beaded male before mentioned, were produced. One of these males was sterile. One of the remaining two was fertile, but

gave no Beaded offspring either in the first generation or in the F_2 generation, although nearly one thousand of his grandchildren were carefully examined. The remaining male was abundantly fertile and had one son exactly like his father in appearance (with a slight nick at the tip of the wings). The rest of his offspring were normal. This son was sterile.

TABLE XXIX

BACK-CROSSES OF F_1 FLIES OF THE CROSS BEADED × PINK TO PINK FLIES OF NORMAL STOCK

	Beaded Pink	Normal Pink	Beaded Red	Normal Red	Exp. Bd. Pink	Exp. No. Beaded R.
F_1 ♂ ×Pink ♀ ..	1	859	504	580	223	282
F_1 ♀ ×Pink ♂ ..	70	114	114	106	84	100

The results of these tests with five of these supposed "cross-over" males show clearly that they were not normal Beaded flies. As said, they might represent mutations, or the dominance of the gene l, or some abnormality. These are mere guesses, but since there are no authentic cases on record in *Drosophila* of crossing over in the male sex in those cases where the mutants dealt with are well known genetically, *i. e.,* since the only apparent cases occur in the Beaded wings and some of the other not well-known and peculiar mutants of *Drosophila,* we are not justified in assuming that such crossing over takes place here.

III. THE EFFECT OF ENVIRONMENTAL CONDITIONS UPON THE PRODUCTION OF BEADED WINGS

A. GENERAL STATEMENT

If we have so far interpreted the evidence correctly we may formulate the following statement as a provisional hypothesis. A gene B′ located in the third chromosome near that for Ebony is directly responsible for the production of Beaded wings. By itself in the homozygous condition, the fly bearing it may have normal wings, though it usually will have wings somewhat Beaded. In the heterozygous condition, it is rarely, though sometimes,

dominant. The conditions so far presented which cause it to be dominant are two. (1) The presence of a gene l in the second chromosome which can not exist in the homozygous condition. (2) The influence, particularly noticeable in the males, of non-chromosomal constituents of the egg from which the individual arose, so that if the mother had been Beaded, the appearance of Beaded wings in her sons would be increased, and if the mother had been normal the appearance of Beaded wings in her sons would be reduced.

Certain facts already brought out (namely, those presented in Tables II and III) show that the tale is not yet told. Our hypothesis does not explain the fact that from definite numbers of eggs laid at different periods in the life of an individual very different percentages of Beaded-winged offspring arise, and these differences do not form a definite series progressing to or from a high percentage as the individual grows older, but are extremely irregular. We have not gained control over this phenomenon, but the evidence we have to present points strongly to the suggestion that the environmental conditions are the final determiners of the percentage of the Beaded-winged offspring. This environmental control might lie in three distinct methods: (1) The destruction of a certain class of offspring by their differential viability. (2) In the case of Table III the results might be explained on the theory that Beaded flies had a shorter life cycle. This supposition has, however, been disproved as follows. Five non-virgin females from Beaded-winged stock and five non-virgin females from normal-winged stock were put together without males in the same bottle. When the offspring began to hatch they were examined daily. During the first three days 73 flies hatched, of which 11, or 15 per cent., had Beaded wings. During the following five days 261 flies hatched, of which 54, or 20 per cent., had Beaded wings. Since I was particular to take Beaded flies several days old as the parents of these Beaded offspring, the experiment shows that if there is any difference in the length of the larval life, that of normal-winged flies is

the shorter. (3) The determination of whether or not a fly of a given germinal constitution shall have Beaded wings. The first of these effects is probably not the significant one, in view of the following facts.

Although as a rule F_1 normal flies give few Beaded offspring, and F_1 Beaded-winged flies relatively many, nevertheless, as has been said, at times normal flies give a high percentage of Beaded offspring and, occasionally, Beaded flies give a low percentage. This can only mean that the dominance of the factor B′ is variable, and considering the large number of times that it shows itself as a recessive, it must be that this varying dominance has a marked effect on the percentage of Beaded-winged offspring that appear.

The possible amount of variation in the environment surrounding a brood of *Drosophila* developing under laboratory conditions is enormous, even when the attempt is made to keep conditions constant. These variations depend upon the exact ripeness of the bananas used as food, the length of time the food has been fermenting, the amount of food and filter paper used, the size of the bottle in which the larvæ are developing, the tightness of the cotton plug, the temperature of the laboratory, etc. Due to these causes there arise very great differences in the relative moisture content and carbon dioxide content. If the food is not properly prepared it may rot instead of fermenting, or it may mould, or the reaction may be in one bottle quite alkaline and in another very acid. A perfect control thus becomes an impossibility, and therefore the experiments to be described must be considered as trials only, and not as decisive tests.

In all the experiments on this subject, Beaded flies of pure stock were mated to normal flies of Wild stock in order to learn the effect of particular environments on the percentage of Beaded offspring in the F_1 generation. On our hypothesis, the pure Beaded flies from stock should be of two kinds, viz., those with the lethal gene l (*i. e.*, B′lB′L), and those without l (*i. e.*, B′LB′L). Correspondingly there should be two types of offspring in the F_1

generation, one of which (B′lb′L) should have a considerably higher percentage of Beaded offspring than the other (B′Lb′L). If it is possible, however, that B′ should be dominant in the heterozygous condition and in the absence of l, then it should also be possible theoretically to produce an F_1 generation every individual of which should have Beaded wings, while those with l as well as B′ (constituting one half the progeny) should have a more extreme form of Beading. In practice it is not usual even under the best of conditions to get more than 40 per cent. of Beaded-winged flies, while, as has been seen, the average amount is about 25 per cent.

B. The Effect of Relative Moisture

Table XXX and Charts 5 and 6 present the data for

TABLE XXX

Percentages of Beaded-winged Flies in the F_1 Generation in Relatively Wet and Dry Bottles. Not Done in Pairs, but Each Bottle Contained Several Pairs

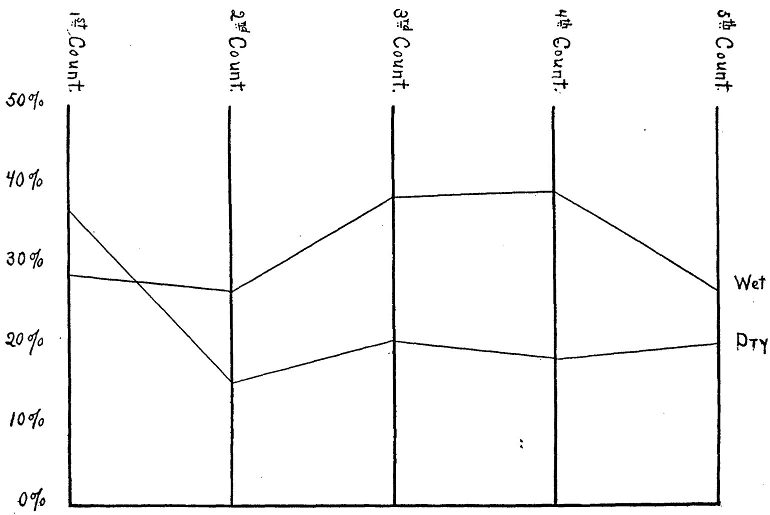
	Dry Bottles				Wet Bottles					
	No. 1		No. 2		No, 3		No. 4		No. 5	
	No. Flies	% Bd.	No. Flies	% Bd.	No. Flies	% Bd.	No. Flies	% Bd.	No. Flies	% Bd.
1st count........	38	31.6	32	43.7	26	26.9	14	28.6	26	30.8
2d count..........	122	14.7	58	17.2	79	32.9	87	20.7	131	26.7
3d count..........	34	20.6	19	21.1	24	41.6	16	37.5	37	37.9
4th count........	33	27.3	34	8.8	39	35.9	17	41.2	43	41.7
5th count........	59	20.3	72	25.0	35	31.4	30	36.7	53	18.9
6th count........	95	10.5	37	35.1						
Total..........	281	17.8	252	24.6	203	33.5	164	28	290	29.3
Total Dry........533	% Bd. 20.5				Total Wet, 657			% Bd. 30.3		

Counts not made every day.

Bottle No. 2 was very dry and the flies very small during time of last two counts.

this test. The parents were put into bottles of similar size with plenty of food. In three of these bottles the food was very wet and from time to time juice was added in sufficient amount to keep the food saturated. The other two bottles were made relatively dry by putting a

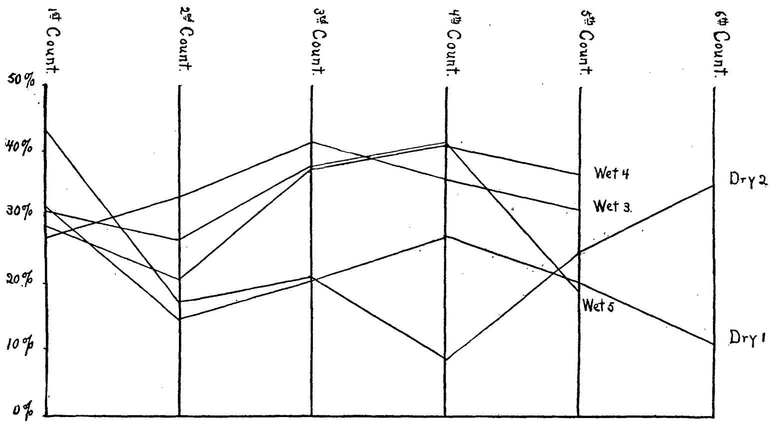
CHART V



Effect of Relative Moisture in Food on Percentages of Beaded-winged Flies in F_1 Generation of Beaded × Wild.

large amount of filter paper into the bottle at night and removing it the following morning. After two or three days of this treatment the bottles were so dry that I did not venture to carry the process farther; the flies from

CHART VI



Effect of Relative Moisture in Food on Production of Beaded Wings, as shown by Individual Bottles.

these dry bottles were rather small and in bottle No. 2, they were extremely small in the last two counts.

From Chart 6, where the records are given of the individual bottles, it will be seen that there is a good deal of irregularity from day to day.

Special attention should be called to the curve of production of bottle 2, which beginning with a high percentage of Beaded offspring gives fewer and fewer for the first four counts (about six days) and then the percentage rapidly mounts again. The offspring given during the last two counts were of surprising minuteness and gave as high a percentage of Beaded individuals as the average of all the bottles on the first day. It has been suggested that it may not be wetness or dryness or any one specific thing that brings out the Beadedness, but conditions that are unfavorable to the organism as a whole, resulting in poor nourishment. It has frequently been

TABLE XXXI

THE INFLUENCE OF ACID, ALKALINE, AND FRESH FOOD ON THE DEVELOPMENT OF BEADED WINGS

	Bd. ♀	Bd. ♂	N. ♀	N. ♂	Per Cent. Bd. ♀	Per Cent. Bd. ♂	Per Cent. Bd. Total
Food Sour							
Mother Beaded; Father normal........	26	40	151	129	14.7	23.8	19.1
Father Beaded; Mother normal........	9	7	60	61	13.0	10.3	11.7
Food Fresh							
Mother Beaded; Father normal........	23	25	85	92	21.3	21.4	21.3
Father Beaded; Mother normal........	54	15	147	164	26.9	8.4	18.2
Food Alkaline							
Mother Beaded; Father normal........	36	41	40	41	46.8	50.0	48.7
Father Beaded; Mother normal........	28	16	57	58	32.9	21.6	27.7

noted that those bottles which gave very tiny flies gave also a higher percentage of Beaded individuals than the bottles whose flies were of average size. On the other hand, the first flies of a brood are almost invariably larger than the later ones, and yet, as has been seen, they are more Beaded. This is a paradox, but the behavior of bottle No. 2 suggests that as a hatch proceeds and the bottle becomes drier, there may be a certain optimum point for the production of normal winged offspring, and

that this point is so low that the flies are poorly nourished for lack of water, though they can survive an even greater water reduction.

It is, perhaps, needless to say that an effort has been made after these experiments to keep the moisture content high and fairly uniform in cases where other environments were being tested.

C. The Effects of Covering with Paraffine the Mouth of the Bottle in which the Flies are Developing

On observing that the proportion of Beaded to Normal offspring was lowered as a hatch continued, it seemed possible that this might be due to one or to both of two causes: (1) The diminishing water content. This matter has already been considered. (2) To a changing carbon-dioxide content. When a brood is first counted the cotton plug that has been for several days in the mouth of the bottle is removed, and in removing the flies the air within the bottle is very apt to be much changed. With this possibility in mind a number of bottles were supplied with food and flies, and after ten days (when the larvæ were beginning to pupate) the parent flies were removed, a little new food put into the bottle and a paraffine cap melted over the cotton so that the bottles were tightly

TABLE XXXII

Counts of Seven Broods which Hatched During Two Periods, the First of Which was Spent in a Bottle Sealed with Paraffine, and the Second in a Bottle Covered with Cheese Cloth. Bottle No. 7 was not Sealed with Paraffine but had been Lightly Stoppered with Cotton

	Bottle 1		Bottle 2		Bottle 3		Bottle 4		Bottle 5		Bottle 6		Bottle 7	
	No. Flies	% Bd.	No. Flies	% Bd.	No. Flies	% Bd.	No. Flies	% Bd.	No. Flies	% Bd.	No. Flies	% Bd.	No. Flies	% Bd.
1st count	75	36	82	40	54	44	51	45	59	29	18	39	87	28
2d count	54	9	32	3	17	6	22	18	36	0	15	13	52	2

Total, first count, 426, per cent. Bd., 36.
Total, second count, 228, per cent. Bd., 6.
Total, both counts, 654, per cent. Bd., 25.

sealed. At the same time other bottles were very loosely covered with a light cotton plug. The bottles remained covered till flies had been hatching for four or five days and then the plugs were removed and the flies counted. The paraffine plugs were not replaced; after carefully renewing the air in the bottles, they were covered with cheese cloth and their brood counted again in four days. The results of this test are given in Table XXXII. The results are striking enough at first sight, but I do not know just what their significance is. They show exactly the same phenomenon that is described earlier and illustrated in Table III. They are more striking than any case I have yet found of the sort, and yet the first inference drawn, viz., that the markedly higher percentage of Beaded flies in the first count is due to these flies having undergone their late development in a "close" atmosphere, must be qualified by the statement that "close" does not refer to the carbon-dioxide content.

At first suspecting this to be the case, I made an apparatus by means of which fresh air could be drawn through a bottle during the entire development of the brood. By this means the carbon-dioxide content could not become very high. In order to prevent drying out, a large amount of food was put into the bottle and the air which was to enter the bottle was first passed through water. The hatching period was prolonged in the cool sink. The results were decisive. One hundred and sixty-nine flies were hatched in the first four days, of which 32 per cent. were Beaded. One hundred and eighty-four flies were hatched in the next four days, of which 10 per cent. were Beaded.

The same flies that were the parents of this brood were in the meanwhile transferred to another bottle, which was covered with paraffine. The first four days of hatching gave 108 flies, of which 15 per cent. were Beaded.

This case shows conclusively that the carbon-dioxide content of the bottles is not the feature of the closed bottles that determines whether or not a fly shall have Beaded wings. It leaves the question still unsettled as

to the effect of moisture, but corresponds to the results obtained in the study of moisture effects.

D. The Effects of Acidity and Alkalinity of the Food

Normally the reaction of food at the time of putting it in the bottles is acid, the degree of acidity depending upon the length of time it has been fermenting. This sourness usually passes gradually away as the larvæ grow older, and by the time a brood begins to hatch the reaction is frequently quite alkaline, unless fresh food has been put recently into the bottle.

On the other hand, if the acidity of the food is neutralized at the beginning with sodium hydrate or carbonate, or if the reaction is made alkaline while yet there remains a good deal of unfermented banana, the acidity will return for a time if not carefully guarded against. Therefore to keep the reaction acid or alkaline is a difficult matter, and requires occasional stirring of the food to make the reaction uniform; this operation is likely to prove disastrous for the developing pupæ.

In the tests here recorded I used food that had been fermenting for one month, so that it had a very acid reaction that lasted till hatching time. For studies of the effect of alkalinity I used food that had been fermenting about one day and mixed with it sodium carbonate, sodium hydrate or ammonia. The results were unsatisfactory and the reaction did not remain constant in spite of my efforts, though on the whole it remained alkaline, and became strongly alkaline, and also slimy towards the end of the experiment, and not a great many flies hatched.

I also used food that had not been allowed to ferment at all, and although I do not know its reaction, it was certainly not so alkaline as the last mentioned, nor so acid as the first. It was soon attacked by mold (Bread mold). I refer to it here as fresh food.

The results are given in Table XXXI, but may be more briefly summarized here.

Of 483 flies raised on sour food, 17 per cent. were Beaded.
Of 605 flies raised on fresh food, 19.3 per cent. were Beaded.
Of 317 flies raised on alkaline food, 38.1 per cent. were Beaded.

In other words, a high percentage of Beadedness came from flies raised on alkaline food, a low percentage from flies raised on acid food, and intermediate amount from flies raised on fresh food.

A careful study of Table XXXI will reveal the curious partial sex-linkage of which I spoke on pages 15 et seq., and here, too, the explanation suggested there seems to apply as in other cases of the sort. It is not a little peculiar that in all of these food tests this phenomenon should have occurred, though I consider this purely a coincidence. In any case, if we can draw any conclusion at all from its appearance, it would only be that the reaction of the food has nothing to do with the occurrence of the phenomenon rather than the reverse.

E. The Effects of Relative Temperatures

No evident effect was produced by rearing the F_1 generation in an ice-chest, but ratios were as varying as when the flies were raised at room temperature. Ratios of 15.4 per cent., 19.2 per cent., 10.3 per cent., 20 per cent. of Beaded offspring are examples of those given by broods raised at low temperatures. The cold does, however, lengthen greatly the larval life and flies were in the case of the brood last mentioned twenty-eight days in hatching. The brood consisted of 312 normal and 77 Beaded-winged flies.

Similar results were obtained in experiments with heat, except that here the larval life was correspondingly shortened and was at times reduced to eight days. It was not found practicable to keep the flies at higher than 30°–33° Centigrade, as they soon died at higher temperatures.

F. The Effects of Darkness

Flies were raised in complete darkness and sister broods in full daylight, but no differences appeared in the offspring. Of 484 flies raised in darkness 30 per cent. had Beaded wings. Of 360 flies raised in the daylight, 29 per cent. had Beaded wings. This experiment seems

to show conclusively that light and darkness do not influence the percentages of Beaded-winged flies.

IV. THE EFFECT OF SELECTION ON THE PRODUCTION OF DIFFERENT TYPES OF BEADEDNESS

Just how much can be accomplished by selection in Beaded stock was one of the first questions that arose. Morgan (1911*a*) describes the origin of pure Beaded stock as having occurred through the selection of Beaded flies in the early generations after its first appearance. He says the first Beaded fly found arose in a culture of *Drosophila* that had been exposed to radium. Mated to his sisters, 1.6 per cent. of the offspring were Beaded. When these Beaded flies were inbred 3 per cent. of the offspring were Beaded. These inbred gave 8.5 per cent. Beaded offspring.

> The same process continued through many generations has finally produced stock that gives in certain cultures nearly 100 per cent. Beaded wings.

In continuing these selection experiments, he says more extreme forms of Beaded wings appeared, and at the time of publishing (March, 1911) he was attempting "to fix some of these extreme variations." While engaged in this work other wing forms arose, most of which are among the best-known mutants of *Drosophila*. Among these are Truncate, Miniature, Rudimentary, Vestigial and Balloon wings, and the Black and Yellow body colors. Most of these forms have been "purified" now and Beadedness never appears in them though it can still be found in Vestigial stock. All of the above-named forms, by the way, with the exception of Truncate and Rudimentary bred true from the start. The Truncate case is not yet published and Rudimentary has proved (Morgan and Tice, 1914) to be due to a single Mendelian factor. The Rudimentary flies were at first self sterile and highly non-viable, and therefore gave peculiar results in breeding tests.

When I first began work with Beaded flies (Sept., 1912) the stock gave 100 per cent. Beaded-winged offspring.

So soon that I did not realize it, nor think to count the generations, I had one stock that gave offspring much more extremely Beaded than the ordinary stock, and this stock is the one on which most of this report is based. About December, 1912, I started one stock bottle to form the basis for a "No selection" test. The parents of this brood were "pure stock Beaded" males and females. The first generation, no normal-winged flies appeared. The generations following were made up by shaking at random from the bottle of the generation before a dozen or two flies into a new bottle.

The second, third and fourth generations gave three normal-winged flies to 325 Beaded. The sixth, 3 normal to 100 Beaded. In later generations I occasionally found normal flies. The stock is in its 27th generation now, the 25th generation having given rise to a large brood of which I counted 541 flies (284 ♀ and 257 ♂), all of which had Beaded wings of a type averaging like those of Figs. 4–6. It is very apparent that the stock is not undergoing any marked change, though I can not guarantee that it would give exactly the same results in other respects as the extreme (selected) Beaded stock that I have used in the linkage tests.

On the other hand, I have not been able thus far to increase the Beadedness of the selected stock beyond a point which it apparently reached many generations ago. The Figs. 1–12 (excepting 2 and 4), which are here reproduced, were made under Dr. Morgan's direction long before I took up the work, and the forms he had drawn then are as extreme as any that I now have.

If this extreme stock be allowed to go without selection for two or three generations, it "reverts" to a less extreme form, from which it can apparently be recovered by one mass selection. I feel confident that in selecting the extreme forms one merely selects a large percentage of individuals that are heterozygous for l, and of course when the stock is not selected for a while, LL forms become relatively more numerous. This would account for all the facts here recorded.

On the other hand, selection for less extreme Beading is also rapidly effective and normal-winged forms appear soon, but this effect soon reaches its limit apparently, and a normal strain or even a strain throwing a high percentage of normals has not yet been obtained. I am not yet certain that it can not be done. I selected in each direction for eleven generations without marked success beyond that here recorded.

V. MUTATION IN BEADED STOCK

A. General Statement

As will be gathered from statements made in the last section, the Beaded stock has been prolific in giving mutations. There has been no especial attempt made to see how many different mutants could be obtained from the stock, and yet a goodly number have appeared. Most of these have been marked types showing little variation and coming out regularly and distinctly in Mendelian proportions in crosses with other types. They have in general bred true from the start without further selection.

A few of these have been of a sort to confuse for a time the study that I have been making, because of their resemblance to certain types of Beaded flies. The criterion in every case as to whether or not a fly was an ordinary Beaded fly or a new "mutant" was its genetic behavior, and the cases to be here described have, with the exception of Stumpy, shown themselves to be due to a single gene conforming in general to those of other well-known mutants of *Drosophila.*

B. Perfect Notched Wings

In the beginning of my work on Beaded wings I thought it might be possible to isolate definite types from the Beaded stock by crossing out to Wild and extracting the F_2 types that appeared; or by back-crossing the F_1 forms to Wild again and extracting new types, etc. Several thousand flies were raised in the hope of accomplishing this, but the "types" found did not breed true, but continued to behave like ordinary Beaded flies, from whose

many original types none were distinguishable. Finally a genuine new "type" appeared, with both wings alike and definitely "notched" (Fig. 13, p. 730). This female which was at sight named Perfect Notched, was mated to Wild. Her ancestry was as follows:

The grandmother came from pure Beaded stock, and the grandfather from Wild stock. Their offspring consisted of 13 Beaded and 69 normal flies.

A Beaded female of this generation was mated to a normal brother and gave 100 Beaded offspring, male and female, and one "perfect notched" female.

This female and her descendants behaved in a very different manner, genetically, than the Beaded stock from which she arose.

She was mated to a Wild male and gave 62 Beaded offspring and 112 normal offspring. Of the Beadeds, 50 were notched in a way resembling the parent and of the 50, 49 were females. Several other peculiar wing types appeared among the remaining 12 Beaded flies of this generation, but did not breed true and were later discarded.

The notched male gave ordinary Beaded and normal offspring and never gave in either the first or later generations any "notched" offspring. He was probably an extreme variant of a common Beaded type (Fig. 4).

Of the normal offspring of the Perfect Notched female four pairs were made up. Seven hundred and forty-nine normal sons and daughters appeared, and no notched.

Of the notched daughters of the perfect notched female, two were mated to normal brothers and two to Wild males. Their progeny was:

	Notched ♀	Notched ♂	Normal ♀	Normal ♂
By normal brothers	53	0	79	69
By wild males	56	0	47	46

Six of the notched females of this generation were mated to normal brothers and gave

Notched ♀	Notched ♂	Normal ♀	Normal ♂
126	0	144	120

At this time, June, it was necessary to leave New York. In traveling, the Perfect Notched stock was lost. Enough

had, however, been done to show definitely the nature of the mutation involved. It is clear that the perfect notched wings owed their appearance to a dominant sex-linked gene, lethal for males. This accounts for the fact that the males are only half as numerous as the females, and none of them notched, while notched and normal females occur in nearly equal numbers. It also accounts for the fact that the normal females of these generations gave no notched offspring.

Other sex-linked lethal genes have appeared from time to time in the crosses of Beaded flies with others, but none of them were dominant, and therefore they made themselves evident only by preventing the development of one half of the males. I have not worked out the inheritance of these cases.

C. Spread Wings

Comment has already been made on the extreme number of wing types that appeared both in the F_1, F_2, and back-cross generations of the cross between Beaded and Vestigial flies. Most of these forms gave results too complex to be analyzed at present. However, among the offspring of a considerable number of the F_1 females there were flies with wings perfectly normal in appearance save that they were held at right angles to the long axis of the body. In all, 60 flies with Spread wings appeared. One of the 60 had wings very slightly Beaded. Some of them were mated together and produced only spread-winged offspring with no sign of Beadedness. Spread-winged males were mated to Pink Black females in order to test the linkage of Spread. (Pink is in the third chromosome group, and Black in the second.) The F_1 generation gave only flies with red eyes, gray bodies and normal wings (neither Spread nor Beaded). In the F_2 generation were Black flies, Gray flies, and Red-eyed flies with normal and with Spread wings, but none of the Pink-eyed flies had Spread wings, though a large number of F_2 Pink normal flies appeared. The Pink-eyed flies were also mated inter se, but no Spread-winged flies appeared in the F_3 generation. This definitely places the gene for

Spread wings in the third chromosome group. Beaded wings have not appeared in the stock bottles of Spread which breeds perfectly true.

D. Stumpy Wings

Recently a new non-lethal sex-linked character has appeared in the offspring of the cross of an F_1 Beaded male to a Wild female. Its nature has not yet been worked out, since only males have thus far appeared. The flies have wings resembling those of Vestigial, save that they are not held at right angles to the body, but in the normal position. Vestigial is not a sex-linked character.

Summary

The character under consideration is that of Beaded wings in *Drosophila ampelophila*. All gradations of form between that of normal wings (Fig. 1) and those shown in Figs. 2 to 12 occur in the stock bottles, though certain selected strains of the stock give no normal-winged offspring.

When a Beaded fly is mated to a fly of a stock not carrying genes for Beadedness in its germ plasm a varying percentage of the F_1 offspring is Beaded. If the male parent is Beaded the majority of the Beaded offspring are usually females; and if the female parent is Beaded, the majority of the Beaded offspring are usually males. A female Beaded fly however gives a larger percentage of Beaded daughters than does a male Beaded fly. This phenomenon is repeated from generation to generation, no matter whether a given Beaded fly has come from a male or female Beaded parent, and this shows that the phenomenon is not caused by a sex-linked gene.

This phenomenon is not caused by non-disjunction of a sex-linked gene, for tests of both the Beaded and Wild stocks showed non-disjunction to be a rare phenomenon. The only explanation suggested was that the male offspring were somewhat influenced to or away from Beadedness by the nature of the cytoplasm that was brought in with the egg, while females were not readily influenced in this way.

A study of the F_2 generation shows that the majority of the normal F_1 offspring differ from the majority of the Beaded F_1 offspring genetically in that normals give fewer Beaded offspring in the F_2 generation than do the Beaded flies.

Beaded wings showed no linkage to any sex-linked character.

Approximately one half of the flies of the F_1 generation of a cross between Beaded flies and flies with characters whose genes were in the second chromosome, showed linkage in the following generation to second chromosome characters, while one half of the flies did not show such linkage. The cases where linkage did not occur gave a slightly lower percentage of Beaded offspring than did those where linkage was present. An explanation of these phenomena is sought in the suggestion that there was in the second chromosome a gene, here called l, that was recessive but that in the heterozygous condition intensified the dominance of another gene, called B′, which was not in the second chromosome. This gene l behaves as a lethal factor preventing the development of any fly that carries it in a homozygous condition.

All of the F_1 offspring of the crosses of Beaded flies by flies with characters caused by genes in the third chromosome showed linkage in the following generation between Beaded wings and the third chromosome characters. This was taken to signify that there was in the third chromosome a non-lethal gene concerned in the development of Beaded wings. This gene was called B′. This gene was shown to be the essential germinal factor in the production of Beaded wings. It is sometimes dominant and sometimes recessive.

The determination as to whether B′ should be dominant or recessive seems to lie in several possibilities: 1st, the nature of the egg cytoplasm; 2d, the presence or absence of the gene l; 3d, the nature of the environmental conditions.

With reference to environmental conditions, it was shown that a larger percentage of the F_1 generation had Beaded wings when the culture was wet than when it was

dry; and more when the food was alkaline than when it was acid. No other environmental factors were discovered which influenced the production of Beaded wings.

Selection of more or less extreme Beaded flies very quickly moves the average Beadedness of the offspring in the direction of the selection, but this selection apparently becomes further ineffective in a very few generations.

Mutation is of very frequent occurrence in the Beaded stock and the new mutants obtained have in most cases shown themselves to be produced under the influence of one normally Mendelizing gene.

I acknowledge with pleasure the kindly interest and suggestions made from time to time by Dr. A. H. Sturtevant and Mr. H. J. Muller. These have been of much assistance to me. My thanks are also especially due to Dr. T. H. Morgan whose advice and criticisms at critical points have never failed to aid in clearing up the situation.

BIBLIOGRAPHY

Baur, E. 1912. Einführung in die experimentale Vererbungslehre.

Bridges, C. B. 1913. Non-disjunction of the Sex Chromosomes of *Drosophila*. *Jour. Exp. Zool.*, Vol. 15.

Hoge, M. A. 1914. The Influence of Temperature on the Development of a Mendelian Character. *Jour. Exp. Zool.* In press.

MacDowell, E. C. 1914. Multiple Factors in Mendelian Inheritance. *Jour. Exp. Zool.*, Vol. 16.

Morgan, T. H. 1911*a*. The Origin of Nine Wing Mutations in *Drosophila*. *Science*, N. S., Vol. 33.

Morgan, T. H. 1911*b*. A Dominant Sex-limited Character. *Proc. Soc. Exp. Biol. and Med.*, Vol. 9.

Morgan, T. H. 1912*a*. The Masking of a Mendelian Result by the Influence of the Environment. *Proc. Soc. Exp. Biol. and Med.*, Vol. 9.

Morgan, T. H. 1912*b*. The Explanation of a New Sex-ratio in *Drosophila*. *Science*, N. S., Vol. 36.

Morgan, T. H. 1912*c*. Complete Linkage in the Second Chromosome of the Male. *Science*, N. S., Vol. 36.

Morgan, T. H. 1912*d*. A Modification of the Sex-ratio and of other Ratios in *Drosophila* through Linkage. *Zts. ind. Abst. u. Vererb.*, Bd. VII.

Morgan, T. H., and Tice, S. C. 1914. The Influence of the Environment on the Size of Expected Classes. *Biol. Bull.*, Vol. 26.

Shull, G. H. 1914.

Sturtevant, A. H. 1913. A Third Group of Linked Genes in *Drosophila ampelophila*. *Science*, N. S., Vol. 37.